REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-99 are in this case. Claims 21-50 and 71-99 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1-20 and 51-70 have been rejected.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 1-20 and 51-70 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiners rejections are respectfully traversed.

With respect to claim 1 and where recited in other claims, the Examiner points out that the phrase "progenitor cells" is uncertain as to meaning and scope. The Examiner states that the difference between progenitor cells and hematopoietic stem cells is uncertain, and it is uncertain as to cells that are progenitor cells and cells that are not progenitor cells. The Examiner further states, that if progenitor cells are any cells capable of transformation into other cells, then the independent claims should require only undifferentiated progenitor cells, and in a dependent claim further define the progenitor cells as undifferentiated hematopoietic stem cells.

As is described in the specification of the instant application the phrase "progenitor cell" refers to a cell, which is the earliest committed cell in a terminal differentiation path. Thus, a hemopoietic progenitor cell is a committed yet immature (not fully differentiated) hemopoietic cell.

As is further described in the specification of the instant application the phrase "undifferentiated hemopoietic stem cells" refers to hemopoietic cells, which are not committed to a differentiation path (page 20 lines 24-26).

Due to the description of such cell types in the specification and the wide use thereof in the art, one skilled in the art would readily recognize, and understand the phrases "progenitor cells" and "undifferentiated hemopoietic stem cells" and the recited embodiments thereof.

35 U.S.C. § 103(a) Rejections

The Examiner has rejected claims 1-20 and 51-70 under 35 U.S.C. § 103(a) as being unpatentable over Naughton et al. in view of Sussman et al. and Stephanopouos et al.

The Examiner states that Naughton et al., disclose the growth of bone marrow stromal cells on a three dimensional matrix, which can be formed from a polymeric material, followed by the inoculation of the stromal matrix with stem cells and maintenance of the stem cells in vitro where proliferation of the cells is maximized to thereby allow long-term maintenance of hematopoietic cells.

The Examiner further states that Sussman et al. disclose a fibrous matrix for cell cultivation. The matrix can be a non-woven fiber sheet with distinct pore volume, pore size and hight, which can be used as a packing in a column and coated with poly-D-lysine.

The Examiner further states that Stephanopolous et al. disclose a cell-culturing reactor having an inlet and outlet for culture medium and containing a macroporous support between the inlet and outlet having pores of a size that allows cells to collect within the pores and oxygen and nutrients to migrate into the pores for consumption by the cells.

The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time of the invention was made to use the matrix of Naughton et al. and the non woven fibrous sheet packed in a column for cell-culture disclosed by Sussman et al. to obtain a flow through reactor having an inlet and outlet as suggested by Sussman et al. and Stephanopoulos et al. since such a reactor would have been expected to provide culturing advantages.

Applicant wishes to point out that Naughton et al. fail to teach or suggest expansion of stem cells under conditions suitable for maintaining such stem cells in a non-differentiated state, whereas the claimed invention is clearly limited to differentiationless stem cell expansion.

Reviewing the experimental data presented by Naughton et al. in Figures 3 and 4, it is clear that the three dimensional system disclosed thereby appears to maintain physiological numbers/levels and distribution of hematopoietic cells. This is in complete contrast with the present invention as claimed in claims 1 and 51, in which

a three dimensional stromal cell bioreactor provides a system for the expansion of transplantable human hematopoietic stem cells, while maintaining an undifferentiated population of stem cells (i.e., differentiationless stem cell expansion).

Evidence that Naughton et al. culture under conditions suitable for differentiation of cells and thus maintenance of physiological numbers/levels and distribution of hematopoietic cells comes from cell typing analysis performed thereby on non-adherent three dimensional long term bone marrow culture (LTBMC). As is illustrated in Table 2 of Naughton et al., such analysis revealed that the LTBMC culture consisted of 60% myeloid cells, 19 % erythroid cells, 11 % lymphoid cells, 4 % macrophages, monocytes and fibroblasts and the rest a population of unidentified blasts. This distribution persisted for the term of culture (i.e., 12 weeks) although the relative percentages of the cell types varied. Thus, macrophages monocytes and fibroblasts released into the medium increased in-time at the expense of the myeloid cells. Similar results were obtained with rat and primate cultures (Column 37, lines 52-67). Consistently, adherent LTBMC analysis revealed that the relative percentage of stromal cells to hematopoietic cells increased with time and that stromal cell proliferation at later periods occurred at the expense of hematopoiesis (Column 38, lines 43-53).

In addition, cytopfluorographic analysis of cellular content of adherent zone 3D LTBMC showed that the cultures (i.e., murine, monkey and human) contained early and late myeloid cells, B and T lymphocytes, megakaryocytes/platelets and monocytes and macrophages;the presence of undifferentiated stem cells was not addressed clearly illustrating that differentiation occurred in the culture described by Naughton et al.

Furthermore as shown in Figure 3, cellular proliferation analysis showed that no expansion of cell numbers occurred under the indicated experimental conditions (Column 39, lines 31-44), illustrating that hemopoietic cell survival rather than cell proliferation are described by Naughton et al.

Altogether, the results presented by Naughton et al. teach the use of a three dimensional bone marrow culture for the maintenance of differentiated bone marrow cells rather than expansion of non-differentiated stem cells, which is the essence of the claimed invention.

It should be noted that Naughton et al acknowledge that the disclosed culturing conditions are permissive to differentiation.

> *"Furthermore, differentiation appears to proceed in a physiologic manner. For example, erythroid, myeloid, lymphoid, macrophagic, and megakaryocytic colonies can continuously arise in the same culture using the systems as taught by the present invention and described below. Stem cell replication in this system can be inferred from the sustained proliferation of committed progenitors".* (Column 21 lines 21-28)

In sharp contrast, the present invention uses plug flow bioreactor in order to maintain hematopoeitic stem cells in a non-differentiated yet proliferative state. Such conditions are neither described nor suggested by Naughton et al. since the object of their invention was to merely to expand a bone marrow derived cell population under conditions which mimic bone marrow physiology.

As is clearly illustrated in the instant specification, the present inventors have demonstrated for the first time that the use of a 3D matrix in a plug flow bioreactor greatly enhances HSC expansion while preventing differentiation of these cell types.

> *"These findings demonstrate that the 3D plug flow bioreactor provides a suitable system for ex-vivo maintenance/expansion of human HSC via superior stromal-stem cell contact and perhaps via stromal cell production of known and/or novel stem cell regulators."* (Page 15, lines 20-23)

Since culturing according to Naughton et al requires differentiation, the use of a plug flow bioreactor is not required nor is it desired (more cell loss) and as such, Naughton et al do not suggest the use of a plug flow bioreactor with their culture and thus would not motivate one of skill in the art to use a flow bioreactor with their culture.

Additional evidence to the lack of motivation comes from the fact that in the 7 years that past since the patent by Naughton et al. issued, not a single article describing

use of a plug flow bioreactor for expansion of HSC has published. If such an approach presented advantages for expansion/differentiation of Hemopoietic cells as taught by Naughton et al., it is highly likely that in a rapidly expanding field such as cell replacement therapy, rapid adoption of such an approach would have been evident both scientifically and commercially.

Thus, one of skills in the art interested in obtaining stem cell expansion would not be motivated to rely on the teachings of Naughton et al. altogether. One interested in differentiationless stem cell expansion would be reluctant to use the teaching of Naughton et al. which disclose the exact opposite, i.e., how to maintain a differentiated cell population. Needless to say that one of skills in the art interested in obtaining differentiationless cell expansion would not be motivated to combine the teachings of Naughton et al. with those of Sussman et al. and/or Stephanopouos et al. because either of these references fails to teach differentiationless stem cell expansion. Also, it is clear that one of skills in the art, based on the teachings of Naughton et al., Sussman et al. and Stephanopouos et al., taken alone, or in any combination, cannot reasonably expect to obtain differentiationless stem cell expansion.

Furthermore, the method described by Naughton et al. involves the seeding of bone marrow cells on a pre-established 3D stromal cell matrix. It is appreciated that since the inoculum used by Naughton et al. also contains stromal cell elements and/or precursors, Naughton et al. cannot, via their experimentation, distinguish between an effect of the 3D stromal cell matrix or of the co-inoculated stromal cells, on the output of hematopoietic cells. This leaves the claim of an effect of the 3D stromal matrix, uninvestigated and questionable.

Thus, yet another difference between the claimed invention and Naughton et al. is that according to the claimed invention stem cells are grown in plug-flow bioreactor in which a three dimensional stromal cell culture has been pre-established.

Hence, it is clear that since Naughton et al. fail to teach differentiationless stem cell expansion, rather Naughton et al. teach maintenance of a differentiated cell population, Naughton et al. create no expectation to succeed in obtaining differentiationless stem cell expansion, using either static bioreactor, as taught by Naughton et al. themselves, or flow through bioreactor, as taught by Sussman et al. and Stephanopouos et al.

It is, therefore, the Applicant's strong opinion that Naughton et al. in view of Sussman et al. and Stephanopouos et al. fail to render claims 1-20 and 51-70 obvious.

Support for Claims Amendments

In view of the above remarks it is respectfully submitted that claims 1-20 and 51-70 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Enc.

Two-months extension fee